CUTICULAR LININGS AND REMODELISATION PROCESSES IN *CRAMBE CRAMBE* (DEMOSPONGIAE: POECILOSCLERIDA)

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Turon, X., Uriz, M.J. & Willenz, Ph. 1999 06 30: Cuticular linings and remodelisation processes in *Crambe crambe* (Demospongiae: Poecilosclerida). *Memoirs of the Queensland Museum* **44**: 617-625. Brisbane. ISSN 0079-8835.

The common Mediterranean sublittoral sponge *Crambe crambe* goes through a resting, non-feeding period with cellular restructuring which may have biological and ecological significance. This red encrusting sponge reproduces in summer and larvae released during July-August. After reproduction, from the end of August until the end of October, some specimens appeared covered with a glassy cuticle, obliterating the ostia and oscula. No water pumping and, hence, no feeding occurs during this stage. At the end of October and during November some specimens displayed a strongly hispid surface, with spicules retaining entangled debris. This hispid form is interpreted as an intermediate stage between the resting phase and the active period. SEM examination of the surface during the non-feeding period confirmed the absence of inhalant orifices and the presence of an acellular cuticle markedly different from the glycocalyx layer associated with the pinacoderm of active specimens. In some individuals, micro-organisms were found adhering to the outer side of the cuticle which were absent from the surface of active specimens. In TEM, the cuticle appeared as a complex 2.5-3µm thick structure made up of three layers: a proximal dense layer (0.06-0.12µm), an intermediate amorphous layer (0.15-0.3µm), and an outer granular layer also of variable thickness (more than 2µm) which progressively disintegrated. Collagen debris appeared between the proximal and intermediate layers. The zone beneath this triple-layered cuticle was either completely devoid of cells or showed scarce degenerating cellular components (mainly from pinacocytes and spherulous cells), and sparse collagen fibrils. The choanosome appeared rather disorganised, with most choanocyte chambers disintegrated, with abundant phagocytosing archeocytes, sclerocytes, spherulous cells, degenerated cells and cell debris. Later in the season the cuticle appeared broken in many places. It was cast off and a new pinacoderm with ostia developed below; filtering activity of the sponges resumed. Spicules, previously protected by the cuticle, were uncovered, giving rise to a hispid phase. Subsequently the emergent spicules were cast off and smoothness of the sponge surface was restored. These changes in sponge cell structure and activity may be explained as reorganisation processes after reproduction, but other causes, such as adverse water temperature, may have similar effects. ❐ *Porifera, aquiferous system reorganisation, cuticle, glycocalyx, external surface, resting stage, SEM, TEM, fine structure, Crambe crambe, Demospongiae, reproduction, Mediterranean Sea.*

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External surfaces of sponges have an important role in the exchange of particles and gases between sponges and the environment. Pinacocytes are the main cells implicated in dermal structures of sponges (reviewed in Simpson, 1984), although spherulous cells can occasionally glide between exopinacocytes and temporarily remain on the surface of the sponge (Uriz et al., 1996; Willenz & Pomponi, 1996). However, non-cellular surface structures have also been described in several species of sponges. An external cuticle has been recorded in Dictyoceratida (Connes et al., 1971; Garrone, 1975; Donadey, 1982; Teragawa, 1986), Verongida (Vacelet, 1971), Chondrosiida (e.g. *Thymosia guerrei* (Rosell, 1988; Boury-Esnault & Lopes, 1985; Carballo, 1994) and *Thymosiopsis cuticulatus* (Vacelet & Perez, 1998)), and Poecilosclerida (e.g. *Microciona prolifera* (Bagby, 1970)). When such a cuticle is present, the exopinacocytes either disappear or lose the ability to capture particles directly from the environment. When they

remain, their feeding depends on particles transferred by archeocytes.

It has been shown in several species of different taxonomic orders that both exopinacocytes and choanocytes produce an external layer of mucopolisaccharides, for example, *Oscarella lobularis* (Lévi & Porte, 1962), *Haliclona elegans*, *Chondrilla nucula* and *Hippospongia communis* (Garrone et al., 1971), *Hemimycale columella* (Willenz, 1981, 1982), *Ceratoporella nicholsoni* and *Stromatospongia norae* (Willenz & Hartman, 1989), and *Myceliospongia araneosa* (Vacelet & Perez, 1998). This glycocalyx is consistent along the surface of the pinacoderm, has a variable thickness and can have the appearance of a cuticle. It plays a role in the adhesion of external particles to cell surfaces, prior to their phagocytosis (Willenz, 1982).

In contrast to the well known function of glycocalyx coats, the nature and biological role of cuticles in sponges remains speculative. Cuticles have been seen as mechanisms that allow isolation of the sponge from the environment for cell repair or reorganisation, or for survival during adverse environmental conditions (Vacelet, 1971; Diaz, 1979). Another possible function is to get rid of harmful epibionts since this cuticle is periodically shed (Connes et al., 1971; Donadey, 1982).

The red, encrusting sponge *Crambe crambe* is a common species in the Mediterranean sublittoral. Its structure, biology, ecology and defense mechanisms are well known from several recent studies (reviewed in Becerro et al., 1997). This sponge reproduces in summer, with larval release occurring from the end of July until the end of August in the NE of Spain (Uriz et al., 1998). Here we describe the existence of a resting, non-feeding stage in *Crambe crambe* and compare its finestructure to that of active individuals.

MATERIALS AND METHODS

FIELD OBSERVATIONS. Field work was undertaken near Blanes (NE coast of Spain). Individuals in a resting stage were clearly discernible *in situ* from contracted normal individuals because their surface appeared glassy and in places showed a translucent, occasionally ridged, film. The extent of the phenomenon was determined by recording the number of individuals with a glassy surface in horizontal transects placed at random on vertical rocky walls (10m long x 1m wide, N=10). The number of specimens reproducing in 1998 was assessed by counting the number of individuals incubating larvae in the same study area. The timing and intensity of reproduction of this species in the area has been assessed regularly over recent years by monitoring both the number of individuals incubating larvae and the abundance of larvae in the water column (Uriz et al., 1998, and unpublished data).

FINE STRUCTURE. Specimens of *Crambe crambe* with different external appearance (normal, contracted, glassy and hispid) were collected from two Mediterranean localities: Calvi, Corsica (in winter 1982) and Blanes, NE coast of Spain (in winter 1993, and summer 1997 and 1998).

For transmission electron microscopy (TEM), specimens were fixed for 5hrs in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (osmolarity adjusted to 980mOsM with saecharose). Samples were washed several times in the buffer solution and postfixed for 90mins in 2% osmium tetroxide (OsO_4) in the same buffer, dehydrated in acetone series and embedded in ERL 4206 according to Spurr (1969). Ultrathin sections, double contrasted with uranyl acetate and lead citrate according to Reynolds (1963) were examined either with a Hitachi H-600 (University of Barcelona) or with an AEI transmission electron microscope (Université Libre de Bruxelles).

To obtain a contrast enhancement of the glycocalyx, ruthenium red was added for some samples (50mg/100ml) in each solution from glutaraldehyde to 70% alcohol (Garrone et al., 1971; Luft, 1971a, b, c; Willenz, 1982; Willenz & Hartman, 1989; Hartman & Willenz, 1990). The fixation time was threefold for those samples and postfixation was performed in the dark (Willenz, 1982).

For scanning electron microscopy (SEM), samples were fixed in a 6:1 mixture of 2% osmium tetroxide (OsO_4) in sea water and a saturated aqueous solution of mercuric chloride ($HgCl_2$) for 90mins (Johnston & Hildeman, 1982). They were then cryofractured in liquid nitrogen, thawed in amyl-acetate at ambient temperature, critical point dried from carbon dioxide, mounted and sputter-coated with gold following standard procedures, and finally examined with a Hitachi-2300 scanning electron microscope (University of Barcelona).

RESULTS

FIELD OBSERVATION. When observed in situ, *Crambe crambe* usually has a clean, smooth surface with conspicuous inhalant and exhalant

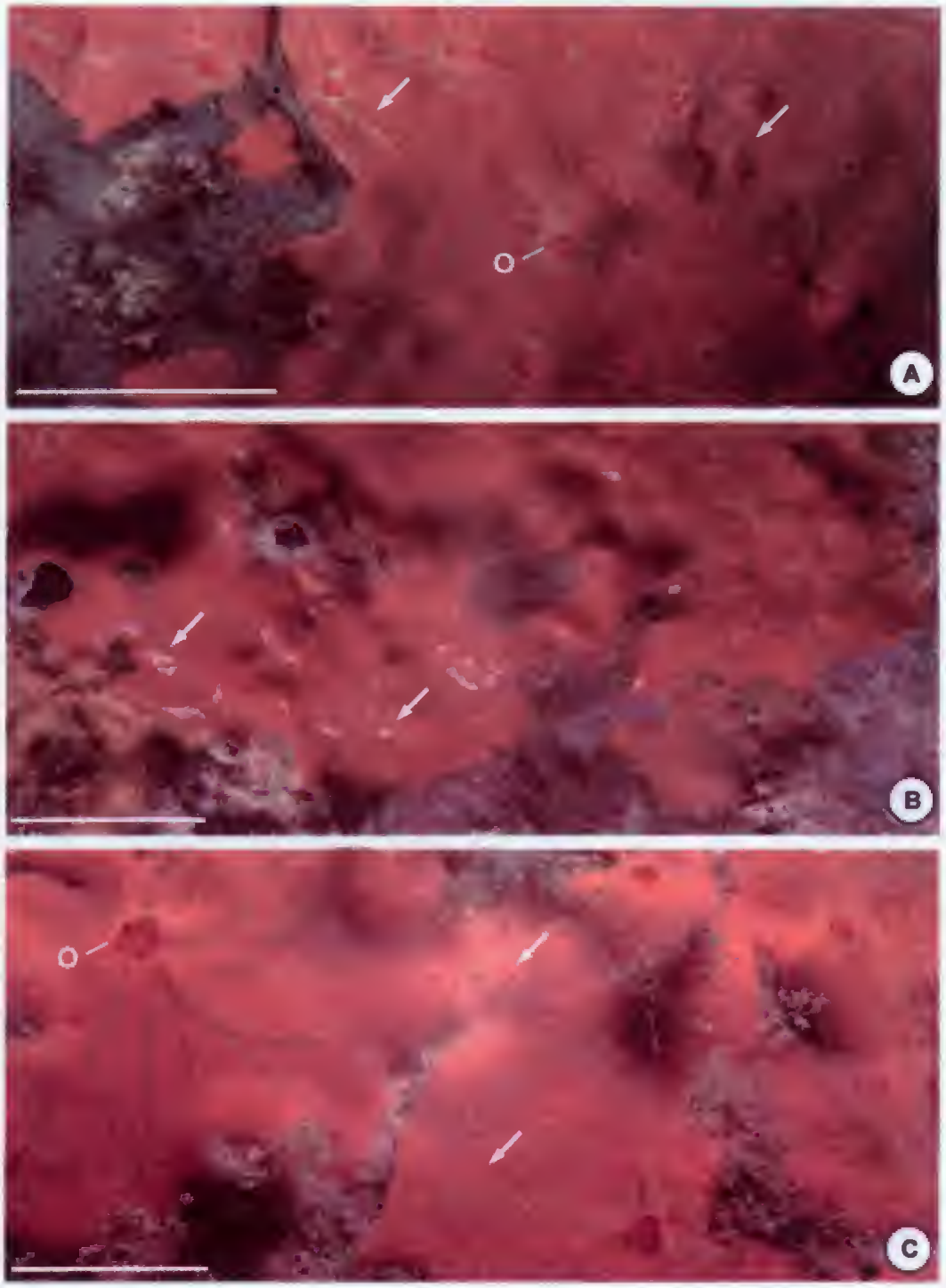

FIG. 1. *Crambe crambe*. A, Active specimen seen in situ. Arrows indicate prominent exhalant canals. O = Osculum. (Scale bar = 5cm). B, Inactive specimen seen in situ. Arrows indicate glassy surface. Oscula are sealed. (Scale bar = 2cm). C, Hispid specimen seen in situ. Arrows indicate debris entangled with spicules. O=Osculum. (Scale bar = 2cm)

orifices and prominent exhalant canals (Fig. 1A). However, in the Blanes littoral, 5% to 20% (in 1997 and 1998, respectively) of the *Crambe* population appeared strongly contracted and with a glassy surface from mid-August to the end of October (Fig. 1B). Specimens were covered by a translucent film. During that stage no inhalant orifices or oscula were perceptible and sponges seem unable to filter water or to feed. In October-November, the surface of some specimens was covered by noticeable amounts of particulate matter and debris (Fig. 1C), contrasting with the usually clean and smooth surface of this species. Upon closer observation, abundant spicules protruded from the surface of these specimens and retained debris. Unfortunately, no data on the percentage of specimens in the hispid phase could be gathered.

FINE STRUCTURE. *Active individuals.* SEM examination of active, water-pumping sponges showed a smooth surface, perforated by abundant ostia (Fig. 2A). No micro-epibionts were present on the sponge surface. The ectosome was rich in collagen fibrils, collencytes and spherulous cells. The exopinacoderm was covered by a dense layer of acid mucopolysaccharides (glycocalyx), 0.25-0.35μm thick, which exhibited a fibrillar structure (i.e. fibrils perpendicular to the cell surface traversed by two more dense bands parallel to the cell surface; Fig. 2B). This structure was enhanced by the ruthenium red staining.

The choanosome was formed by small choanocyte chambers with 7-13 choanocytes, as seen in histological sections, archeocytes, sclerocytes, spherulous cells and endopinacocytes. A central cell was often visible in the choanocyte chambers (Galera et al., in press). Choanocytes had a basal nucleus which often protruded from the cell, only covered by the cell membrane. They produced a conspicuous glycocalyx around the base of the flagellum (Fig. 2C). Transverse sections also revealed acid mucopolysaccharide secretions filling the space between collars, as well as extending from the flagellum (Fig. 2D). Conversely, the endopinacocytes did not appear to produce any glycocalyx.

Inactive individuals. SEM examination of the sponge surface during the non-pumping period confirmed the absence of ostia and revealed the presence of an acellular cuticle (Fig. 3A-B), as well as the almost total absence of an exopinacoderm. In TEM, the cuticle appeared as a complex 2.5-3μm thick structure, comprising three layers (Fig. 3C, E): a proximal dense layer (0.06-0.12μm), an intermediate amorphous layer (0.15-0.3μm), and an outer granular layer (2μm or more) in different stages of disintegration. Collagen debris appeared intermingled between the proximal and the intermediate layers. The region below this triple-layered cuticle was either devoid of cells or showed scarce degenerating cellular components (mainly pinacocytes and spherulous cells), and sparse collagen fibrils. The choanosome appeared rather disorganised; some choanocyte chambers were still recognisable, while others were severely disintegrated (Fig. 4A). Archeocytes, sclerocytes, spherulous cells, degenerated cells and cell debris were abundant, and phagocytosis by archeocytes was common (Fig. 4B).

At more advanced stages, the cuticle appeared broken in many places. Field observation and SEM images indicated that the cutiele was being cast off, with a new pinacoderm developing below. Spicules, previously covered by the cuticle, became exposed, giving rise to a hispid phase (Fig. 3D). New functional ostia developed on the dermal membrane and water pumping activity resumed. We assume that protruding spieules were expelled, as the sponge surface recovered its normal smooth appearance.

DISCUSSION

The resting stage of *Crambe crambe*, characterised by the presence of a well developed cuticle covering a disorganised choanosome, is followed by the formation of a new pinacoderm, the rejection of the cuticle and reorganisation of the choanosome. This sequence may be related to a reconstruction of the sponge canal system after larval release. Similar ultrastructural changes have been considered to be a reorganisation process after damage or reproduction in *Suberites massa* (Diaz, 1979). In *Crambe crambe*, however, these morphological changes do not occur in all specimens every year. This phenomenon was particularly intense during the last two years (1997 and 1998), although it had been occasionally observed before 1997. In 1997 it affected only an estimated 5% of the population at one time whereas 50% of individuals were engaged in sexual reproduction (authors, unpublished data). In 1998 the percentage of specimens going through reproduction was below one third of that in previous years and the number of released larvae was exceptionally low (3-5 larvae per m^3 of water vs. more than 200 larvae per m^3 of water seen in previous years).

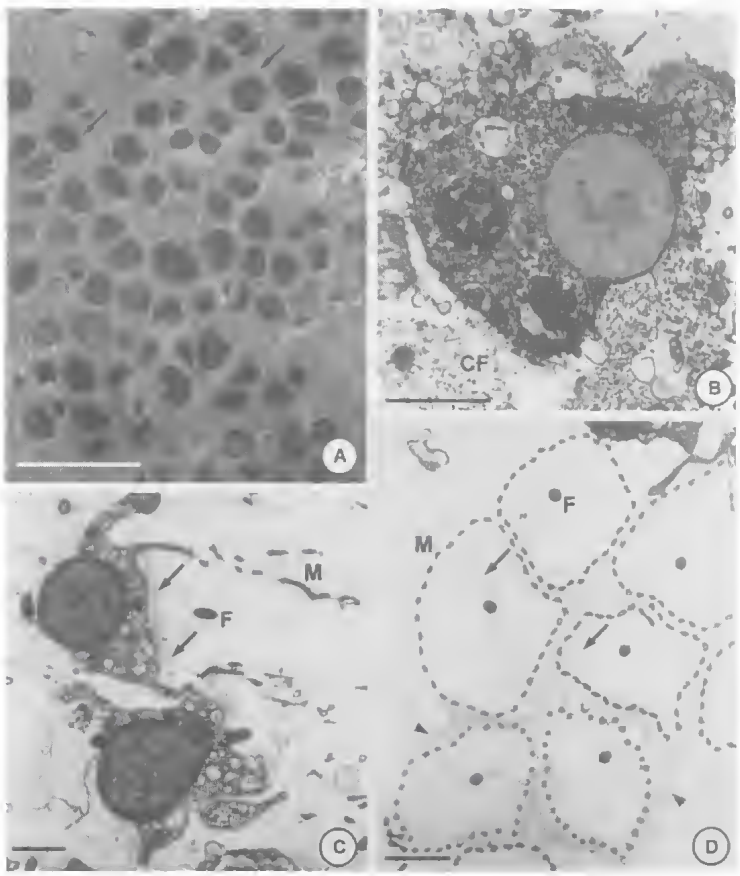

FIG. 2. *Crambe crambe*. A, SEM view of active specimen shows smooth surface and abundant wide open ostia (arrows). (Scale bar = 200μm). B, Active stage. Cross section of pinacocyte covered with dense glycocalyx (arrow). CF = collagen fibrils. (Scale ba r = 1μm). C, Active stage. Cross section of choanocytes with basal nucleus (N); arrows indicate well developed glycocalyx; F = flagellum; m = collar microvilli. (Scale bar = 1μm). D, Active stage. Transverse section through periflagellar collar. Muchopolysaccharide material (arrowheads) fills the space between collars and forms lateral expansions of the flagellum (F); m = collar microvilli. (Scale bar = 1μm).

Nevertheless, up to 20% of the population went into resting stages and related morphological changes. Environmental factors other than reproduction may be triggering this phenomenon. During summer 1997, the water temperature in the Mediterranean area was the highest of the past 25 years. In contrast, summer 1998 was characterised by a sharp drop of water temperature in the first half of August (from 25°C-19°C), just at the onset of larval release, caused by deep cold water mixing with shallow warm waters. It may be possible, therefore, that the resting stages develop not only in response of remodelisation following the process of sexual reproduction, but also as an effect of water temperature abnormalities.

There are no essential differences between the structure and thickness of permanent cuticles (Vacelet & Perez, 1998), and transient cuticles reported here and by Vacelet (1971) and Donadey (1982). Both permanent and transient cuticles are directly in contact with collagen fibrils and seem to replace the pinacoderm. When the transient cuticle is cast off, some collagen fibrils are also lost, along with cellular debris. Most sponges appear to be able to form a cuticle, for instance, *Aplysina* spp, *Microciona prolifera* and *Stelletta grubei* (Bagby, 1970; Vacelet, 1971; Boury-Esnault, 1975; Simpson et al., 1985). Other sponge species that exhibit the glassy appearance of resting stages mostly in autumn-winter, are *Spongia officinalis, S. agaricina, Ircinia fasciculata, Cacospongia scalaris, C. mollior* (authors, personal observation). Factors triggering resting stages are still poorly known, but seem to be related to adverse environmental conditions, such as insufficient water current (Vacelet, 1971), or strong water temperature variations (this paper), remodelisation after sexual reproduction (this paper), or injury (Diaz, 1979).

Most cuticles are described to last for relatively short periods of time. The biological functions of transient cuticles remains speculative. In keratose sponges, cuticles are periodically shed possibly to eliminate harmful epibionts (Vacelet, 1971; Donadey, 1982). Permanent cuticles were only rarely reported (Vacelet & Perez, 1998). Their role remains unknown, but they may help maintain the internal milieu of sponges containing a high density of symbiotic, extracellular bacteria, and separate it from the surrounding water.

Sponges isolate themselves from their

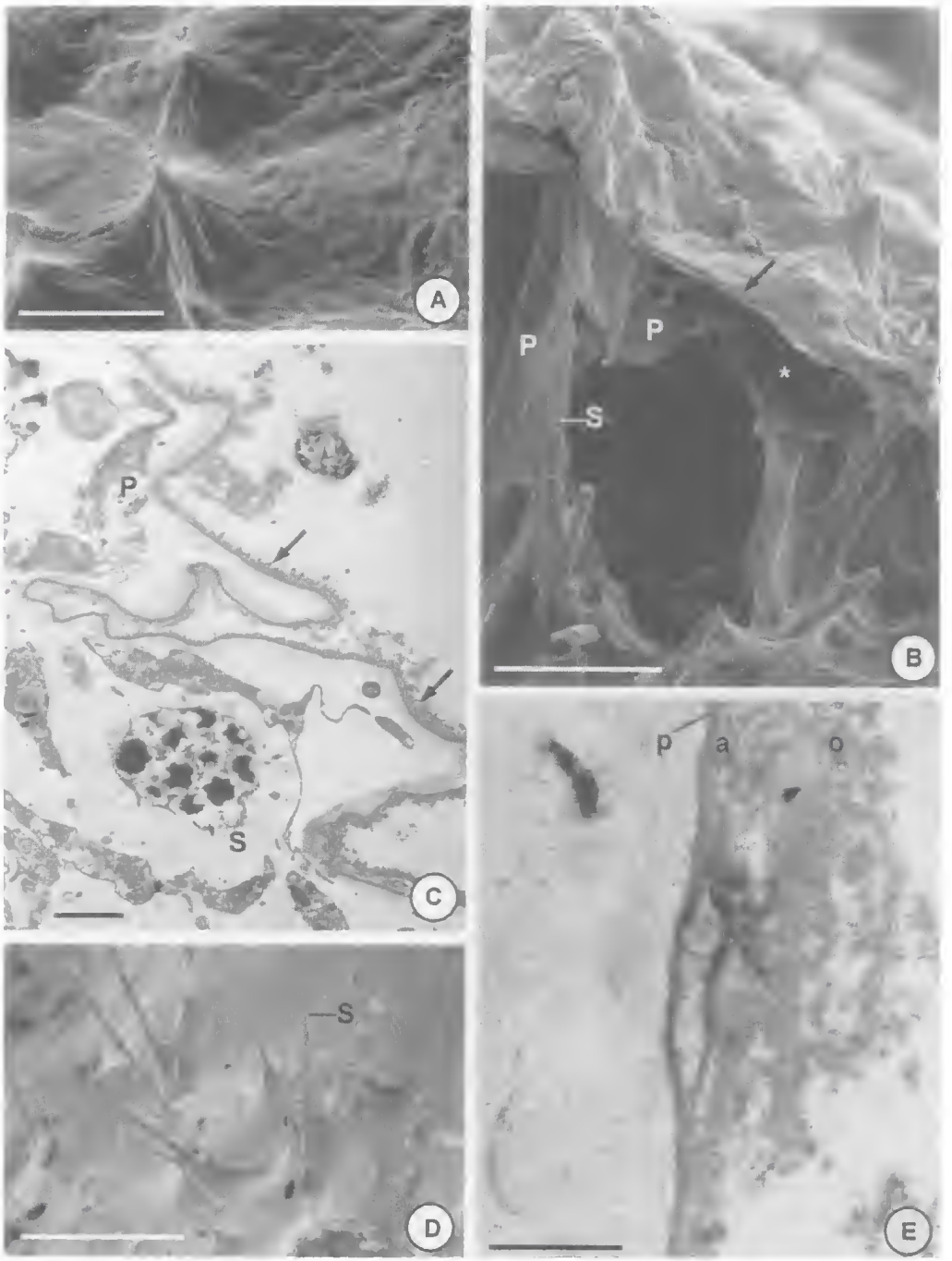

FIG. 3. *Crambe crambe*. A, Resting stage. Sponge surface devoid of ostia and covered by acellular cuticle. (Scale bar = 50μm). B, Resting stage. Cryofracture shows large empty space (asterisk) underneath cuticle (arrow). Fragments of pinacocytes (P) are attached to spicules (S). (Scale bar = 50μm). C, Resting stage. Cross section through cuticle (arrows) with both degenerating pinacocytes (P) and spherulous cells (S). (Scale bar = 10μm). D, Hispid stage. Detail of sponge surface with protruding spicules (S). (Scale bar = 100μm). E, Resting stage. Detail of a cross section through cuticle with triple layer. p = proximal dense layer; a = amorphous intermediate layer; o = outer layer. (Scale bar = 1μm).

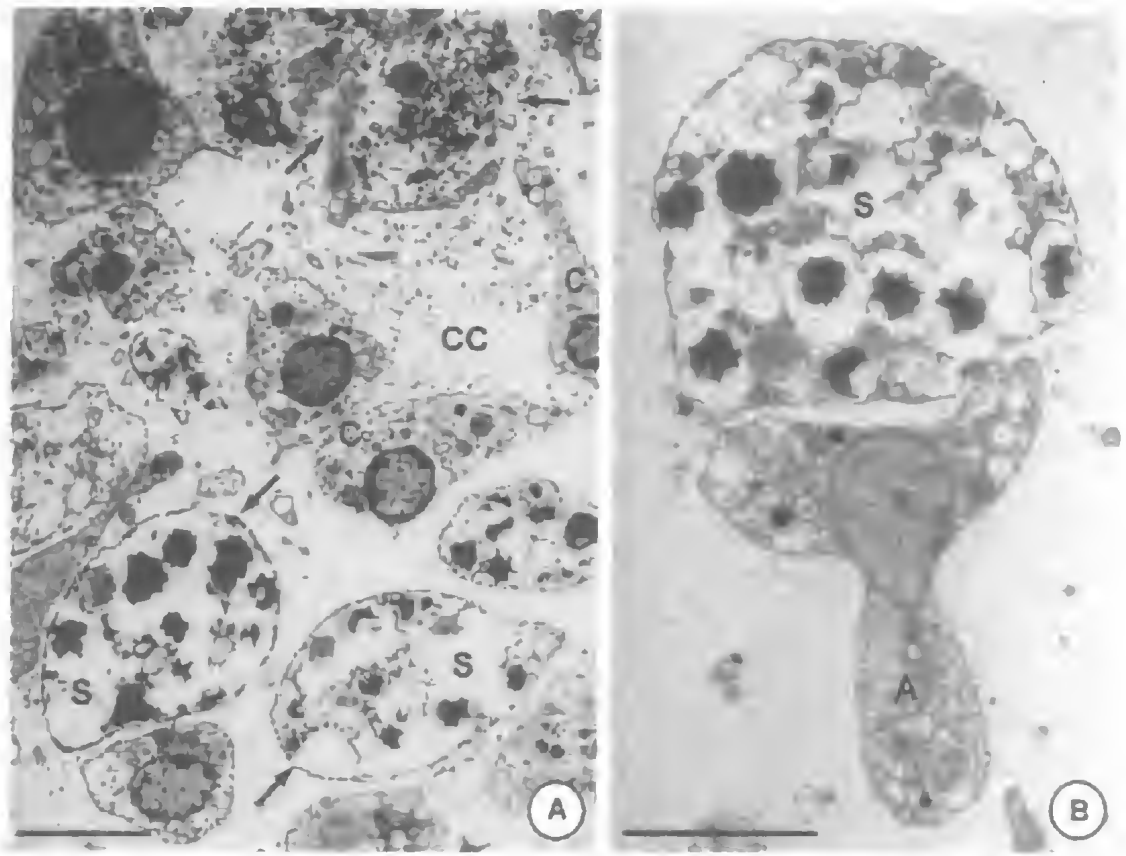

FIG. 4. *Crambe crambe*. A, Resting stage. Choanosome with disintegrated choanocyte chamber (cc) and degenerating cells (arrows); C = choanocytes; S = spherulous cell. (Scale bar = 10μm). B, Resting stage. Degenerating spherulous cell (S) in early stage of phagocytosis by archeocyte (A). (Scale bar = 10μm).

environment on many occasions. They deposit condensed collagen fibrils around harmful inhabitants such as Polychaeta, Cirripedia or Amphipoda (Connes, 1967; Sube, 1970; Connes et al., 1971; Uriz, 1983). The presence of micro-organisms adhering to the transient cuticle of resting specimens of *Crambe crambe* supports the protective role of this structure, isolating the sponge from the environment. In active specimens, the surface is usually free of bacteria (Becerro et al., 1994), owing to spherulous cells containing antimicrobial metabolites which cross the pinacoderm (Uriz et al., 1996). The chemical composition of these 'resistant' barriers is still unknown, but it is likely to be of a proteinaceous nature because collagen debris is visible in some places in the proximal layer. Furthermore, as shown here, it reacts similar to collagen and spongin if subjected to different stains (Vacelet, 1971).

The production of a dense cuticle allowing the sponge to become temporarily isolated from the environment may be a more widespread protective mechanism in sponges than previously thought (Bagby, 1970; Vacelet, 1971). The formation of temporary glassy pellicules is not restricted to sponges. It has also been described from colonial ascidian species belonging to different families (Turon, 1988, 1992), where it was interpreted as a product of a periodic rejuvenation process when the filtering thoraxes of the zooids are being replaced. Glassy cuticles are also reported in some cnidarian species (Garrabou, 1997) where they have been interpreted as protective structures to overcome adverse conditions (summer). Temporary isolation through production of an acellular cuticle may, therefore, be a common mechanism among soft, sessile invertebrates, allowing for survival during periods of adverse conditions or internal reorganisation.

ACKNOWLEDGEMENTS

This research was funded by projects Biomark PL961088 from the European Union (MAST-III program), DGCYT, PB94-0015, CICYT MAR95-1764 from the Spanish Government and 1997SGR-00084 of the 'Generalitat de Catalunya'. We express our gratitude to D. Bay for welcoming one of us (Ph.W.) at STARESO, Calvi, and for providing optimal diving and laboratory conditions. We thank Dr. M. Buscema for assistance in SCUBA diving and M. Doriaux for advice and technical help with EM fixation.

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